



Attorney's Docket No. 8151-195944

## PATENT

### IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Hanley, Jr. et al.  
Appl. No.: 09/560,288  
Filed: April 27, 2000  
For: METHOD FOR PRODUCING HUMAN  
INTERVERTEBRAL DISC CELLS

Group Art Unit: 1647  
Examiner: Hayes, R.C.

July 29, 2004

### DECLARATION UNDER RULE 132 OF DR. HELEN GRUBER

I, Dr. Helen E. Gruber, declare and state:

1. That I received my B.S. degree in pre-medicine from the University of Idaho in 1969, my M.S. degree in cell biology, radiation biology and microbiology from Oregon State University in 1974, and my doctorate in cell biology from Oregon State University in 1976. I am presently a faculty member, interdisciplinary PhD program, Department of Biology, at the University of North Carolina at Charlotte, and adjunct professor, Greenville Hospital System/Clemson University Biomedical Cooperative. Also, I have 100 refereed scientific articles in print and have co-authored 11 book chapters. A copy of my CV appears as Exhibit A.
2. That I am a co-inventor of United States patent application Serial No. 09/560,288 filed April 27, 2000 entitled *Method for Producing Human Intervertebral Disc Cells*.
3. That I have read the Office Action dated May 11 2004 and have considered the examiner's position that "The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims." I respectfully disagree.

4. In United States Patent No. 6,080,579, a parent patent of the present application, of which I am a co-inventor, there is disclosed and claimed a method for propagating human intervertebral disc cells. The claims of this application carry the process of the '579 patent one step further in that the disc cells propagated using the method of the '579 patent are used to treat human disc diseases. More specifically, in a preferred embodiment of the claimed method, the surgeon first removes healthy disc tissue from a disc that does not show degenerative changes using an open procedure or a percutaneous procedure (note in particular claim 48). The patient's own disc cells are grown in tissue culture as described in the patent specification. These cells are then provided to the surgeon who places them into a debrided segment of degenerating disc. Over time these cells and their descendents would form appropriate building blocks of disc tissue in the implanted site. A schematic illustration of this procedure is shown in Figure 1 of the patent application.

5. I next note that the Examiner states "[t]he issue remains that the current claims cannot work in adolescents or adults wherein annulus and/or cells no longer exist." Contrary to the examiner's statement, adolescents and adults both have annulus disc cells. The human cells that would be cultured *in vitro* are cells from the annulus. The type of human cells obtained from expansion of disc tissue *in vitro* which are used in the claimed therapeutic composition are cells from the annulus. There is detailed discussion and examples (Note examples 4 and 5, for instance) of the use of annulus cells in the patent application. In my opinion, the patent application provides sufficient guidance for one skilled in the art to provide the claimed composition using disc cells from the annulus.

6. I also note the examiner states that "the specification fails to define what population of disc cells to use or how" and "the type of matrix made by disc cells is dependent on the environment these cells are placed within and on the type of disc cells used, which the claims fail to fully define." The cells to be used to replace annulus cells in the disc are annulus cells derived from a healthy disc site. As noted in previous responses, the notochordal cell population disappears from the disc, and the annulus cells from the inner region populate the central portion of the disc. Therefore, it is most reasonable and effective to use annulus cells in disc cell

therapy.

7. In addition the examiner states that "disc cells are a heterogeneous population of cells with different intrinsic variations in their response to mechanical stimuli, with distinct biosynthesis capabilities, where what constitutes a success regenerative process is unclear within the art, and in which the instant problems in generating a potential successful "treat[ment of] human disc diseases." Although this statement may be correct regarding detailed elements of gene expression for specific genes, we feel that annulus cells implanted into a diseased disc will readily meet the mechanical and structural needs of the implantation site as the cells integrate into the nearby matrix. We have found successful integration with this method when we used the animal model of the sand rat for autologous disc cell implantation. Therefore, it is logical to predict the same response in the human disc.

8. Having shown and claimed the use of annulus cells in the therapeutic compositions of this invention, it is my opinion that it would not require undue experimentation to make and use the invention as claimed.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Helen E. Gruber  
Dr. Helen E. Gruber

July 29, 2004  
Date

**CURRICULUM VITAE****1. PERSONAL**

**NAME:** Helen Elizabeth Gruber, Ph.D.

**BIRTHDATE:** November 6, 1946

**BIRTHPLACE:** Wallace, Idaho

**CITIZENSHIP:** U.S.A.

**SOCIAL SECURITY NUMBER:** 519-58-0241

**BUSINESS ADDRESS:** Orthopaedic Research Biology  
Department of Orthopaedic Surgery  
Carolinas Medical Center  
PO Box 32861  
Charlotte, North Carolina 28232  
Tel: 704-355-5658; Fax: 704-355-2845  
e-mail: hgruber@carolinas.org

**MARITAL STATUS:** Single

**2. EDUCATION AND TRAINING**

**UNDERGRADUATE:** University of Idaho; Moscow, Idaho;  
B.S., Pre-Med; 1969

**GRADUATE:** Oregon State University; Corvallis, Oregon;  
M.S.; 1974; Cell Biology, Radiation Biology, Microbiology

Oregon State University; Corvallis, Oregon; Ph.D.; 1976; Cell  
Biology, Radiation Biology, Zoology

**3. POSTDOCTORAL TRAINING**

NIH Fellow; Radiation Research Lab, University of Iowa; Iowa City, Iowa; 1976-1978

**4. MILITARY SERVICE:** None**5. ACADEMIC APPOINTMENTS**

Nov 2002 - Present	Research Group Director, Dept. of Orthopaedic Surgery, Carolinas Medical Center, Charlotte, N.C.
June 1999 – present	Faculty member, Interdisciplinary PhD in Biology program, Univ. N.C. Charlotte
Aug 1997 - Present	Adjunct Professor, Greenville Hospital System/Clemson University Biomedical Cooperative, Clemson, S.C.
July 1994- June 2005	Graduate Faculty appointment, University of North Carolina at Charlotte, Charlotte, North Carolina
March 1994- present	Adjunct Professor, Department of Biology, University of North Carolina at Charlotte, Charlotte, North Carolina

January 1994-present	Senior Scientist and Director of Research Histology Baxter Orthopaedic Research Laboratory Department of Orthopaedic Surgery Carolinas Medical Center, Charlotte, North Carolina
July 1991-November 1993	Research Scientist III Cedars-Sinai Medical Center Los Angeles, California
July 1991-November 1993	Associate Researcher II, Department of Pediatrics University of California at Los Angeles Los Angeles, California
July 1991-November 1993	Associate Faculty UCLA Inter-campus Medical Genetics Training Program
July 1989-1991	Associate Researcher I, Department of Pediatrics University of California at Los Angeles Los Angeles, California
1986-1991	Research Scientist II and Director, Skeletal Dysplasia Morphology Laboratory; Department of Pediatrics and Medical Genetics Birth Defects Center, Cedars-Sinai Medical Center Los Angeles, California
1986-1989	Assistant Researcher IV, Department of Pediatrics University of California at Los Angeles Los Angeles, California
1984-1986	Assistant Professor of Research Medicine University of Southern California School of Medicine Department of Medicine, Division of Nephrology Los Angeles, California
1981-1984	Research Fellow, University of Southern California School of Medicine, Division of Nephrology Los Angeles, California (Dr. Shaul G. Massry)
1978-1981	Research Instructor of Medicine University of Washington Stationed at V.A. Medical Center, Tacoma, Washington (Dr. David J. Baylink)
1976-1978	Postdoctoral Fellow, Radiation Research Lab, University of Iowa, Iowa City, Iowa (Dr. J.W. Osborne, advisor)
1974-1976	Research Assistant, Oregon State University Corvallis, Oregon (Dr. David L. Willis, supervisor)
1972-1973	Northwest College and University Association for Science Fellow, Battelle Northwest Laboratories, Richland, Washington (Ph.D. Research conducted under Dr. James C. Hampton)
1970-1972, 1973	Teaching Assistant, General Biology, Department of General Science, Oregon State University, and

## 6. HONORS

- 1984 Listed in Who's Who in Frontier Science and Technology, 1st Ed.
- 1985 Invited Speaker, International Symposium on Magnesium and its Relationship to Cardiovascular, Renal and Metabolic Disorders, February 14, 1985, Workshop on Magnesium and Bone Disorders
- 1986 Appreciation Award, Volunteer Teaching, Griffin Avenue School, Los Angeles, California, June 18, 1986
- 1988 Appreciation Award, Volunteer Teaching, Griffin Avenue School, Los Angeles, California, June 20, 1988
- 1989 Recipient of Departmental Tribute for Distinguished Service, Department of Physical Therapy, University of Southern California, May 1989
- 1989-1990 Ad Hoc Reviewer for Veterans Administration Merit Review Board for Endocrinology
- 1999 Host mentor laboratory, "Implantation methodologies for autologous disc cell therapy". Alpha Omega Alpha Honor Medical Society - funded Student Research Fellowship for David Martin, summer, 1998.
- 2001 Best Basic Science Poster Award, The Cervical Spine Research Society annual meeting. Tenascin in the human intervertebral disc: Alterations with aging and disc degeneration. H.E. Gruber and E.N. Hanley.
- 2001 Kaye E. Wilkins Scientific Poster Award. Pediatric Orthopaedic Society of North America annual meeting. Histologic features of physeal bar formation in physeal fractures. J. Wattenbarger, H.E. Gruber
- 2002 Wattenbarger, JM, A. Marshall, **H. Gruber**, R. Meyer : The role of the basement plate in physeal bar formation. AOA Residents Conference Award. Poster presentation at the Amer. Orthopaedic Association annual meeting, Victoria, B.C. June 1, 2002.

## 7. PUBLIC AND COMMUNITY SERVICE ACTIVITY

- 1986 Appreciation Award, Volunteer Teaching, Griffin Avenue School, Los Angeles, California, June 18, 1986
- 1988 Appreciation Award, Volunteer Teaching, Griffin Avenue School, Los Angeles, California, June 20, 1988
- 1990 Volunteer Teacher, Griffin Avenue School, Los Angeles, California
- 1993 Host laboratory for summer student in NIH-funded Minority High School Student Research Apprenticeship Program, Modoupe Akinloye, (worked on histology cartilage project July-August 1993)

- 1994. Host laboratory and supervisor for Scientist-Teacher-Exchange-Program for local high school science teacher Sharron Mottisinger, July-August
- 1995 Host laboratory and supervisor for teacher Angela Ireland. NIH-funded "Precollege Research Opportunities for Minorities in Health Science Education", A. Harver, UNCC, P.I.
- 1995 Science Fair Judge, Holy Trinity Catholic Middle School, Charlotte, N.C. 12/9/95
- 2002 - Editorial Advisory Board Member, the journal Spine.
- 2002 - Editorial Board Member, Biotechnic & Histochemistry

## **8. MEMBERSHIPS IN PROFESSIONAL AND SCIENTIFIC SOCIETIES**

American Association for the Advancement of Science  
 American Federation for Clinical Research  
 American Society for Bone and Mineral Research  
 American Society for Cell Biology  
 Society for Experimental Biology and Medicine  
 Society for Diagnostic Ultrastructural Pathology  
 Society for Quantitative Morphology  
 Electron Microscopy Society of America  
 Orthopaedic Research Society  
 International Society for Molecular Morphology  
 North American Spine Society

## **9. ACADEMIC AND PROFESSIONAL APPOINTMENTS/COMMITTEES**

### **A. GRADUATE STUDENT COMMITTEE SERVICE**

Ar-tyan Hsu, Ph.D. completed August 1989; University of Southern California, Department of Physical Therapy; dissertation title: "Trabecular architecture and finite element analysis of the human calcaneus"

Kim O'Connor, Ph.D. completed December 1993; Department of Physical Therapy, University of Southern California; research on the effect of weight bearing on cartilage and joint function

Andrew Nelson, Ph.D. candidate; Department of Anthropology, University of California at Los Angeles; research in progress on morphology of bones of early primates

Carl Fisher, Jr. UNCC Dept. of Biology, master's candidate. Completed 8/5/96 "Effect of the insulin-like growth factors and bone morphogenetic protein on cell division and matrix production by human intervertebral disc cells". H. Gruber, advisor and research director.

Bhaloo Desai, Ph.D. Postdoctoral trainee with my laboratory 8/95 through 3/99. Studies on human disc cells in vitro.

Committee member for Bhavini Desai, Ph.D. candidate, Dept. of Biology, U.N.C. Charlotte, 1999 – present.

## **B. EDITORIAL ACTIVITIES**

- |           |   |
|-----------|---|
| 1986-1992 | Editorial Board, Society for Experimental Biology and Medicine  |
| 1989-1993 | Editorial Board, Quest - Science for Health, Cedars-Sinai Medical Center Research Institute Publication |
| 1995-1997 | Editorial Board, Journal of Dental Research   |
| 2002 -    | Editorial Advisory Board Member, the journal "Spine".   |
| 2002.-    | Editorial Board Member, "Biotechnic & Histochemistry".  |

## **C. COMMITTEES**

- |              |   |
|--------------|---|
| 1986-1993    | Cedars-Sinai SSB Laboratory Committee   |
| 1987-1989    | Cedars-Sinai Medical Center Research Committee  |
| 1988-1993    | Cedars-Sinai Bioscience Library Task Force  |
| 1989-1993    | Cedars-Sinai Medical Genetics Laboratory Quality of Service Committee   |
| 1990-1992    | Co-chair, Fellowship Evaluation Program (Mentor Committees)   |
| 1990-1992    | Chair, Medical Genetics Fellow's Journal Club   |
| 1991         | Academic Consultant for Rhone-Poulenc Rorer, FDA Advisory Committee Meeting, July 23-24, 1991, Bethesda, Maryland       |
| 1992         | Chair, Medical Genetics-Birth Defects Center 1991-92 Faculty Retreat, January 10, 1992                                  |
| 1992         | Cedars-Sinai Medical Genetics Staff/Management Job Evaluation Committee   |
| 1992-1993    | Associate Faculty, NIH Medical Genetics Intercampus Training Grant, UCLA  |
| 1992-1993    | Strategic Planning Action Committee - Task Force on UCLA faculty retention  |
| 1998-99      | Mentoring committee member for Mark Tenholder, M.D., Research Resident  |
| 1998-present | Research Review Committee, Carolinas Medical Center   |
| 1999         | "Cannon 2000: a working group of biomedical research faculty", May-June, 1999.  |
| 2000         | Member, Education and Research and Carolinas HealthCare Foundation Advisory Committee for the 2000 United Way campaign. |
| 2002         | Institutional Animal Care and Use Committee, Carolinas Medical Center   |



## 10. LECTURES AND PRESENTATIONS; INVITED PRESENTATIONS AND OTHERS 1994 - PRESENT

"Cartilage: articular and epiphyseal", Orthopaedic Surgery Basic Science Conference, February 14, 1994

"Tendon and ligament", Orthopaedic Surgery Basic Science Conference, March 14, 1994

"Effects of low maternal calcium intake on the maternal and offspring skeleton", May 2, 1994, Calcium Group Noon Meeting, School of Dentistry, University of North Carolina at Chapel Hill.

"New developments in orthopaedic surgery", panel member, Orthopaedic Surgery Basic Science Conference, May 9, 1994.

"Research studies on human intervertebral disc cells", presented to the Dept. of Biology, University of North Carolina at Charlotte, Dec. 9, 1994.

"Bone and hormones", Orthopaedic Surgery Basic Science Conference, Apr. 17, 1995.

"Bone remodelling", Orthopaedic Surgery Basic Science Conference, May 15, 1995.

"Bone development and growth", Orthopaedic Surgery Basic Science Conference, June 12, 1995.

"Cartilage and disc", Orthopaedic Surgery Basic Science Conference, October 16, 1995.

"Inflammation and infection", Orthopaedic Surgery Basic Science Conference, Jan. 15, 1996.

"Skeletal embryology, growth and remodeling", August 19, 1996, Orthopaedic Surgery Basic Science Conference.

"Skeletal dysplasias", Sept. 16, 1996, Orthopaedic Surgery Basic Science Conference

Invited keynote speaker: "Perspectives on the skeletal dysplasias", N.C. Medical Genetics Assoc. Meeting, Dec. 5, 1997.

"Peripheral nerves", Jan 12, 1998, Orthopaedic Surgery Basic Science Conference

Coordinated Orthopaedic Surgery Basic Science Conference on the skeletal dysplasias, 9/21/98

"Tissue engineering for autologous disc cell therapy". Cannon Seminar Series, 9/9/98.

"Nerves", Orthopaedic Surgery Basic Science lecture, Jan 10, 2000

"Infection and inflammation", Orthopaedic Surgery Basic Science lecture, Feb. 14, 2000

"Basic features of bone cell function in health and disease. Invited platform presentation at Medfest 2000: Continuing Medical Education Conference for Primary Care Physicians and Specialists, Blacksburg, VA, May 5-6, 2000

Orthopaedic Surgery Basic Science Lecture: Jan. 14, 2002: Nerves

Orthopaedic Surgery Basic Science Lecture: Feb. 11, 2002: Inflammation and infection

Orthopaedic Surgery Basic Science Lecture: Oct 14, 2002, Osteoblasts and bone formation and osteoclasts and bone resorbing factors

Orthopaedic Surgery Basic Science Lecture: Nov 11, 2002, Osteoblasts and osteoclasts in metabolic bone disease.

Orthopaedic Surgery Basic Science Lecture: Dec. 9, 2002, The physis and physal fractures.

Orthopaedic Surgery Basic Science Lecture: Nov. 10, 2003: Cartilage and Disc

Orthopaedic Surgery Basic Science Lecture: Jan. 12, 2004: Peripheral Nerves.

Orthopaedic Surgery Basic Science Lecture: February 9, 2004: Inflammation and Infection

## **11. RESEARCH SUPPORT**

### **Funded:**

"Investigation of osteopenia and spontaneous fractures in an animal model", University of Southern California Faculty Research and Innovation Fund, \$11,000, 7/1/85-6/30/86

"The influence of low calcium intake on skeletal changes associated with pregnancy and lactation", National Dairy Council, \$32,680, 1/1/86-6/30/87

"Skeletal changes in offspring: Relation to maternal calcium intake and post-weaning diet", Cedars-Sinai Biomedical Research Support Grant, \$5,000, 9/1/86-8/31/87

"Purchase of a critical point dryer and sputter coater: Shared scanning electron microscopy support equipment for Pediatrics, Pathology and Nephrology", Cedars-Sinai Biomedical Research Support Grant, \$6,211, 2/87-3/88

"The skeletal dysplasias", National Institutes of Health (Co-Investigator), D. L. Rimoian, M.D., Ph.D., Principal Investigator, Years 4 and 5, 1989-1990 and 1990-1991

"Rescue and subsequent engraftment of osteogenic cells by bone marrow transplantation: Application to reversal of porcine cyclosporin or corticosteroid-induced osteopenia", Sandoz (Co-Investigator), F.R. Singer, M.D., Principal Investigator, Direct costs \$37,968, 10/1/91-9/29/92

"Assessment of the bone-forming activity of parathyroid hormone analogues", Loma Linda Veterans Association for Research and Education (Sub-contract), David Baylink, M.D. and Sandy Hilliker, Ph.D., Principal Investigators, \$6,750, 10/1/91-9/30/92

"The skeletal dysplasias", National Institutes of Health (Co-Investigator), D.L. Rimoian, M.D., Ph.D., Principal Investigator, Morphology Section, \$19,135, 5 year program project, 12/1/91-11/30/92

"Morphologic evaluation of effect of gallium on the rat osteoclast", (Principal Investigator), Fujisawa Pharmaceutical Co., \$20,000, 8/1/91-12/31/92

"Expanded histomorphometric characterization of bone biopsies from the Baylink/Chesnut calcimar osteoporosis study", (Principal Investigator), Rhone-Poulenc Rorer, \$25,000, 1/1/92-12/31/92

"Assessment of the bone-forming activity of parathyroid hormone analogues", Loma Linda Veterans Association for Research and Education (Sub-contract), David Baylink, M.D. and Sandy Hilliker, Ph.D., Principal Investigators, \$7,500, 3/15/92-3/14/93

"Polylactic acid foam efficacy in spinal fusion - a canine animal model", West Coast Spine Institute/UCLA (Sub-contract), Craig Callewart, M.D., Principal Investigator, Direct costs \$24,473, 7/1/92-12/31/93

"Rescue and subsequent engraftment of osteogenic cells by bone marrow transplantation: Application to reversal of porcine cyclosporin", Sandoz (Co-Investigator), F.R. Singer, M.D., Principal Investigator, Direct costs \$19,970, 11/1/92-4/30/93

"Studies in bone regeneration: Osteogenesis, osteoconduction, and osteoinduction", VAMC West Los Angeles (Sub-contract), T.A. Miller, M.D., Principal Investigator, Direct costs \$2,663, 8/1/92-1/31/94

"The skeletal dysplasias", National Institutes of Health Program Project (Co-Investigator), D.L. Rimoin, M.D., Ph.D., Principal Investigator, Morphology section - \$24,060, Year 7 of program project, 12/1/92-11/30/93

"The skeletal dysplasias", National Institutes of Health Program Project (Co-Investigator), D.L. Rimoin, M.D., Ph.D., Principal Investigator, Direct costs \$8,288, 12/01/93 - 11/30/94; \$8,619.

"Comparison of long term effects of surgical approaches to the acetabulum". O.B. Tabor, Jr., M.D., principal investigator; co-investigators: M.J. Bosse, M.D., J. F. Kellam, M.D., S. H. Sims, M.D., H. E. Gruber, Ph.D. Winkler Orthopaedic Research Fund. 11/28/94 - 5/27/95; \$32,548.

"Research histology studies", summer 1995. To support research for Mr. George Manousos, a year 3-4 PreMed student at UNC-Chapel Hill. \$3700., Charlotte-Mecklenburg Health Services Foundation, Inc.

"Summer studies in the nutrient modulation of disc cell function." To support summer 1995 research activities for Sharon McDaniel, a Queen's college senior who will continue research studies on disc cell culture. \$3700. Charlotte-Mecklenburg Health Services Foundation, Inc.

"Development of soft tissue bioprosthesis". W.D. Holder, Jr., M.D., G. Sonnenfeld, Ph.D., D. H. Hayes, M.D., B. Gordon, D.V.M., P. Watterson, M.D., H. Gruber, Ph.D. (20% effort, H. Gruber). direct costs, \$1,130,000 for 1994 - 1998. CMHA Health Services Foundation (Charlotte, N.C.) and Reprogenesis Corporation (Dallas, Tx).

"Morphologic and biomechanical joint studies in a patient with extensive rheumatoid arthritis". H.E. Gruber and R. Peindl. Smith Arthritis Foundation. 2/1/95 -12/31/96. \$1,950.

"Effect of the insulin-like growth factors and bone morphogenetic protein on cell division and matrix production by human intervertebral disc cells". Charlotte-Mecklenburg Health Services Foundation, Inc. H. E. Gruber. \$12,000. 7/1/95 - 6/30/96.

"Assessment of reamer system mechanical loads and resultant histopathology as factors affecting intramedullary pressurization". R. Peindl, M. Bosse, H. Gruber, et al. Orthopaedic Trauma Association. 11/95-10/96. \$18,365.

"*In situ* PCR hybridization: Detection of the expression of the small proteoglycans". H. E. Gruber and E. N. Hanley, Jr. Charlotte-Mecklenburg Health Services Foundation, Inc., summer 1996, \$3,700.

"Utilization of the sand rat for *in vivo* and *in vitro* studies of the intervertebral disc". H. E. Gruber and E. N. Hanley, Jr. Winkler Orthopaedic Fund, \$15,000, May 1, 1996 - April 30, 1997.

"Technician support for utilization of the sand rat for *in vivo* and *in vitro* studies of the intervertebral disc"; H. E. Gruber and E. N. Hanley, Jr., Charlotte Mecklenburg Health Services Foundation, 10/1/96 - 6/30/97, \$12,251.

"Summer support for a student to work on the project 'Utilization of the sand rat (*P. obesus*) for *in vivo* and *in vitro* studies of the intervertebral disc' ", H. E. Gruber and E. N. Hanley, Jr., summer 1997, \$3700

"Development of soft tissue bioprosthesis". W.D. Holder, Jr., M.D., G. Sonnenfeld, Ph.D., D. H. Hayes, M.D., B. Gordon, D.V.M., P. Watterson, M.D., H. Gruber, Ph.D. (20% effort, H. Gruber). \$526,000 extension through Dec. 1999. CMHA Health Services Foundation (Charlotte, N.C.) and Reprogenesis Corporation (Dallas, Tx).

"Cytosensor Microphysiometer equipment grant for studies of the human intervertebral disc cell in vitro", H. E. Gruber, Molecular Devices Corporation, \$29,750, 3/19/97

"Utilization of the sand rat for *in vivo* and *in vitro* studies of the intervertebral disc". H. E. Gruber and E. N. Hanley, Jr. Winkler Orthopaedic Fund, \$15,000, May 1, 1997 - April 30, 1998.

"Autologous disc cell transplantation in the sand rat (*Psammomys obesus*).", H.E. Gruber and E. N. Hanley, Jr.: (Graduate student project). Charlotte Mecklenburg Health Services Foundation, Inc. \$12,000 for one year. Funded on 6/17/97

"Characterization of sulfate metabolism and programmed cell death in human disc cells in vitro", H.E. Gruber, E. N. Hanley, Jr. and B. J. Desai. \$50,000, North American Spine Society; 1/1/98- 12/31/98

"Tissue engineering for autologous disc cell therapy in a small animal model." H. E. Gruber, and E. N. Hanley, Jr., \$95,000, National Medical Technology Testbed, 1 year beginning 11/15/97

"Programmed cell death and sulfate metabolism in cervical and lumbar human intervertebral discs". H.E. Gruber and E. N. Hanley, Jr. \$30,000, Cervical Spine Research Society. 1/1/98 - 12/31/98

"Implantation methodologies for autologous disc cell therapy". Alpha Omega Alpha Honor Medical Society - funded Student Research Fellowship for David Martin; Gruber lab is host lab; supply money: \$500 in addition to stipend to David. Summer, 1998

"Confocal microscope for the Cannon Research Center". \$250,000. N.C. Biotechnology Center. Awarded 6/15/98. C. Halberstadt, K. Burg, and H.E. Gruber

"Development of a bone cement implant for drug delivery". Fumio Tokita, W.K. Ramp, H.E. Gruber, R.D. Peindl: Charlotte-Mecklenburg Health Services Fdn., \$17,100. 7/1/98 - 6/30/99.

"Support of a breeding colony and adult colony of sand rats (*Psammomys obesus*) for studies of disc degeneration". H.E. Gruber. \$30,000. Charlotte-Mecklenburg Health Services Foundation. 9/1/98 - 8/31/00.

"Programmed cell death and sulfate metabolism in cervical and lumbar human intervertebral discs." H.E. Gruber and E. N. Hanley. The Cervical Spine Research Society. Year 2 competitive renewal. \$30,000 (which includes \$4,500 indirect costs). 1/1/99 - 12/31/99.

"Tissue engineering for autologous disc cell therapy in a small animal model." Year 2 competitive renewal. H.E. Gruber. National Medical Technology Testbed. \$86,786. (15% indirect costs) 4/22/99 - 4/21/00.

"Tissue engineering for the intervertebral disc with the sand rat model". H.E. Gruber and E.N. Hanley, North American Spine Society. 1/1/00 -12/31/00. \$42,830.

"Factors associated with physeal fracture healing and physeal bar formation", M. Wattenbarger, R. Meyer, H.E. Gruber, A. Marshall, Health Services Foundation, \$15,249. 9/1/00-8/31/01

"In vivo response to cross-linked polyethylene using a rat model". A. Marshall, H. Gruber, R. Meyer, T. Fehring. Winkler Orthopaedic Fellowship. \$16,982. 2001

"Human intervertebral disc cell attachment, proliferation and matrix production in vitro: Experimental studies with selected cell carriers for tissue engineering". H.E. Gruber, E.N. Hanley, Jr. North American Spine Society. 1/1/01 -12/31/01. \$50,000.

"Clinical, demographic and experimental characteristics of degenerative disc disease: A retrospective pilot study of patient and control data, disc histopathology and cell responsiveness". H.E. Gruber, D. Heiner, J. Patt, E.N. Hanley. \$45,000. Winkler Orthopaedic Research Fund. 2/11/02 – 12/31/03.

"Magnesium deficiency: Effect on bone and mineral metabolism". R. Rude, H.E. Gruber, B. Mills. NIH – Natl. Institute of Diabetes & Digestive & Kidney Diseases (Nutrient Metabolism Program). 2/1/02 – 1/31/05. Total grant amount: \$302,417.

Summer orthopaedic biology research intern, \$3,700. H.E. Gruber. Charlotte-Mecklenburg Health Services Foundation. 3/1/02-8/31/02

"Proposal for development of polymer rigid inorganic matrix material for orthopaedic trauma uses". J. Kellam, H.E. Gruber, R.A. Meyer, Jr. A.O. \$85,000. 6/1/02 – 12/31/04

"Does disc cell death increase as adjacent vertebral endplates undergo sclerosis?" H.E. Gruber and E.N. Hanley, Jr. A.O. \$30,000. 3/1/04-2/28/05.

## 12. Patents:

"Method for producing human intervertebral disc cells". E.N. Hanley, Jr., and H.E. Gruber. Patent No. 6,080,579. Issue date June 27, 2000.

## 13. BIBLIOGRAPHY

### A. SCIENTIFIC ARTICLES IN PRINT (REFEREED)

1. 1974      **Gruber, H.E.**, Rosario, B.: "Variation in eyespot ultrastructure in Chlamydomonas reinhardi (ac-31)." J. Cell Sci. **15**:481-491
2. 1976      **Gruber, H.E.**, Nachtwey, D.S.: "Cell cycle dependent X-ray induced death in Chlamydomonas reinhardi." Radiat. Res. **66**:288-302
3. 1976      **Gruber, H.E.**, Nachtwey, D.S.: "X-ray induced division delay in Chlamydomonas reinhardi." Radiat. Res. **66**:303-309
4. 1977      **Gruber, H.E.**, Rosario, B.: "Ultrastructural variations in the flagellar apparatus of Chlamydomonas reinhardi (ac-31)." J. Submicrosc. Cytol. **9**:103-113
5. 1978      **Gruber, H.E.**: "X-ray and proton-induced ultrastructural changes in dividing Chlamydomonas reinhardi." Radiat. Res. **73**:137-159
6. 1978      Oberley, L.W., Bize, I., Sahu, S., Leuthauser, S., **Gruber, H.E.**: "Superoxide dismutase activity of normal murine liver, regenerating liver, and H6 hepatoma." J. Natl. Cancer Inst. **61**:375-379
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- 1998 Pfeiffer, L.S., Wattenbarger, M., Meyer, R., **Gruber, H.**, Easley, M.: Effect of interposed periosteum in an animal physal fracture model. Residents' Day Research Presentations, Dept. of Orthopaedic Surgery, May 15, 1998.
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- 1999 Gutteridge, D.H., **Gruber, H.E.**, Kermode, D.G., Worth, G.K.: Paget's disease with primary hyperparathyroidism: Gender distribution in 30 cases, and prediction of skeletal response to parathyroidectomy in 18. Bone. 24 (no. 5, suppl): 51S.
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- 1999 **Gruber, H.E.**, Coleman, S.S., Hanley, E.N.Jr.: In vitro rescue of human disc cells from apoptosis. Platform presentation, North American Spine Society, Oct., 1999, Chicago
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- 2000 **H.E. Gruber** and J.A. Ingram: Invited speaker at the North Carolina Society of Histopathology Technologists, March 31, 2000: Practical approaches to bone and cartilage histology
- 2000 J. Meek, **H. Gruber**, B. Gordon, P. Marshburn: The effect of GnRH analogue pretreatment along with oxidized regenerated cellulose (Interceed) upon post-surgical adhesion formation in the rabbit. Platform presentation at the 26<sup>th</sup> Scientific Meeting of the Society of Gynecologic Surgeons, Feb. 29, 2000, New Orleans.
- 2000 **H.E. Gruber**, S. Coleman, E.N. Hanley, Jr.: Anti-apoptotic effects of cytokines on human intervertebral disc cells in vitro. Poster presentation at the April 7, 2000 N.C. Tissue Engineering Interest Group meeting. Research Triangle Park.
- 2000 **H.E. Gruber**, E.N. Hanley, Jr.: Selective in vitro attachment patterns are used by human disc cells from the annulus in response to different culture microenvironments. Platform presentation, Cervical Spine Research Society, 11/30/2000, Charleston, S.C.
- 2001 **H.E. Gruber** and E.N. Hanley, Jr.: Cell shape modulates differential proliferative potentials in human intervertebral disc cells in vitro. Poster presentation, Amer. Acad. Orthopaedic Surgeons, 68<sup>th</sup> annual meeting, San Francisco, Feb 28-Mar 4, 2001
- 2001 JM Wattenbarger, L. Phieffer, **H. Gruber**: Histologic features of physeal bar formation in physeal fractures. POSNA, May, 2001 meeting. Received Best Poster award.
- 2001 J.M. Wattenbarger, L. Phieffer, **H. Gruber**: Histologic features of physeal bar formation in physeal fractures. Growth Plate Conference, June, 2001.
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- 2001 **Gruber, H.E.**, Leslie, K.P., Johnson, T.L., Ingram, J.A., Phieffer, L., Banks, D., Hanley, E.N.Jr. Autologous disc cell implantation in a small animal model. North American Spine Society 16<sup>th</sup> annual meeting, Seattle, poster presentation, 10/31/01.

- 2001 Rude, R.K., **Gruber, H.E.**, Wei, L., Frausto, A., Mills, B. Magnesium deficiency: Effect on bone and mineral metabolism in the mouse. Poster presentation at the annual meeting of the Amer. Soc. For Bone and Mineral Research. J. Bone Mineral Res. 16, suppl. 1, abstract SA312, p S278, 2001.
- 2001 Gruber, H.E. and Hanley, E.N. Jr.: Autologous disc cell implantation in a small animal model. 95<sup>th</sup> annual meeting, Interurban Orthopaedic Society, Oct. 5-6, Charlotte.
- 2001 **Gruber, H.E.**, Ingram, J., Hanley, E.N.: Tenascin in the human intervertebral disc: Alterations with aging and disc degeneration. Cervical Spine Research Society 29<sup>th</sup> annual meeting, Monterey, CA Nov 29-Dec 1, 2001. Poster presentation. Received 1<sup>st</sup> place as Basic Science Poster.
- 2002 JM Wattenbarger, L. Phieffer, **H. Gruber**: Histologic features of physeal bar formation physeal fractures. Amer. Acad. Orthopaedic Surgeons, poster presentation, 69<sup>th</sup> annual meeting, Feb. 13-17, 2002, Dallas.
- 2002 **Gruber, H.E.**, Gregg, J.: Sub-chondral bone and bone resorption in the TMJ: Is there a role for anti-resorbing agents such as 17- $\beta$ -estradiol? Platform presentation, Second Scientific Meeting of the TMJ Association, Joint and Muscle Dysfunction of the Temporomandibular Joint, May 6-8, 2002, Bethesda, MD.
- 2002 Wattenbarger, JM, A. Marshall, **H. Gruber**, R. Meyer: The role of the basement plate in physeal bar formation. AOA Residents Conference Award. Poster presentation at the Amer. Orthopaedic Association annual meeting, Victoria, B.C. June 1, 2002.
- 2002 **Gruber, H.E.**: Intervertebral disc degeneration and autologous disc cell implantation. Dept. of Biology seminar, Univ. N. C. at Charlotte, April 26, 2002.
- 2002 **Gruber, HE**, Hanley, EN: Autologous disc cell implantation in a small animal model. International Society for the Study of the Lumbar Spine, platform presentation, May 15, Cleveland.
- 2002 **Gruber, HE**, Hanley EN, Leslie K, Ingram, J.: Human intervertebral disc cells: Selected cell carriers for tissue engineering. Poster presentation, North American Spine Society, Oct 29 – Nov 2, 2002 Montreal.
- 2002 Hanley, E.N., **Gruber, HE**, Phieffer, L., Banks, D., Coldham, G., Leslie, K., Ingram, J. Cell-based tissue engineering strategies for intervertebral disc therapy: Autologous cell implantation in a small animal model. Cervical Spine Research Society. Platform presentation. Miami, Dec. 5, 2002.
- 2003 Marshall, A, **Gruber, HE**, Wattenbarger, JM: The role of the basement plate in physeal bar formation. Platform presentation, Amer. Academy of Orthopaedic Surgeons, New Orleans, LA, Feb. 5-9, 2003.
- 2003 **Gruber, H.E.**, Gordon, B., Williams, C., Hanley, E.N. Jr.: Bone mineral density of lumbar vertebral end plates in the aging sand rat spine. International Society for the Study of the Lumbar Spine, 2003 meeting in Vancouver, CA, May 13-17, 2003. Poster presentation.
- 2003 Valerin, M, Brennan, MT, **Gruber, H.**, Padilla R, Ullrich C, Lockhart, PB. Magnetic resonance imaging as a diagnostic tool for pulpal status. Poster presentation, April 29-May 4, 2003 at the American Academy of Oral Medicine annual meeting in Tucson, AZ.
- 2003 Rude, R.K., Gruber, H.E., Norton, H.J., Wei, L.Y., Frausto, A., Mills, B.G.: Increased release of substance P and TNF-alpha underlie osteoporosis induced by Mg restriction in the rat. Poster presentation, American Society for Bone and Mineral Research.

Minneapolis, September 19-23, 2003. J Bone Mineral Metabolism 18(suppl. 2), p S155, 2003.

- 2003 **Gruber, H.E.**, Leslie, K., Norton, H.J., Heiner, D., Patt, J., Hanley, E.N. Jr.: Clinical, demographic and experimental characteristics of degenerative disc disease: Ethnic differences. Dec 11-13, 2003 meeting of the Cervical Spine Research Society at Scottsdale, AZ. Poster presentation.
- 2004 **Gruber, H.E.**, Ingram, J.A., Leslie, K., Hanley, E.N. Jr.: Cellular, but not matrix, immunolocalization of SPARC in the human intervertebral disc: Decreasing localization with aging and degeneration. Poster presentation, Orthopaedic Research Society, Mar. 7-10, 2004. San Francisco.
- 2004 **H.E. Gruber**, E.H. Sage, S. Funk and E.N. Hanley, Jr.: Targeted Deletion of the SPARC Gene Accelerates Disc Degeneration in the Aging Mouse. Poster presentation, 26<sup>th</sup> annual meeting of the Amer. Soc. for Bone and Mineral Res., Seattle, WA, Oct 1-5, 2004.
- 2004 Rude, RK, **Gruber, HE**, Norton, HJ, Wei, LY, Frausto, A, Kilburn, JM. Effect of dietary magnesium reduction to 25% of nutrition requirement on bone and mineral metabolism in the rat. Poster presentation, 26<sup>th</sup> annual meeting of the Amer. Soc. for Bone and Mineral Res., Seattle, WA, Oct 1-5, 2004.

#### **F. MISCELLANEOUS ARTICLES AND PUBLICATIONS**

- 1972 King, Keith I., Pribble, H.J., **Gruber, H.E.**: "The pond as a unit of study." Laboratory manual utilized in GS 101, General Biology, Department of General Science, Oregon State University, 1972
- 1990 **Gruber, H.E.**: "The bone biopsy: A window for viewing the skeleton." Quest, Science for Health, Cedars-Sinai Medical Center 2 (1), summer 1990, p. 9
- 1992 **Gruber, H.E.**: "Donor dollars: Providing a new look at cell structure." Quest, Science for Health, Cedars-Sinai Medical Center 3 (3), 1992, pp. 36-37
- 1992 **Gruber, H.E.**: "Take the drudgery out of typing references." Society for Ultrastructural Pathology Newsletter 15:3-4, October 1992